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## STUDIES IN PYROPHILOUS FUNGI—I. THE OCCURRENCE AND CULTIVATION OF PYRONEMA

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(WITH PLATES 9-12, CONTAINING TWENTY-ONE FIGURES)

To the collector it is a well-known fact that there are numerous species of fungi which are known only on burnt places. While some of these forms may occur under other conditions, such occurrence is so rare as to have attracted comparatively little attention. Many popular reasons have been offered by individuals in explanation of these facts, such as the elimination of competition in the destruction of the higher plants, the presence of carbon in the soil, and that these forms really occur in other habitats and escape detection, but none of these reasons is sufficient to explain the occurrence of at least one of the plants in question. That these fungi do not occur on burnt places simply because the competition of the higher plants has been eliminated is shown by the fact that they do not, as a rule, occur on bare soil which has not been burned over. My own observation has also shown that carbonaceous materials are not necessary to the life of some of the pyrophilous fungi, and we must look for other explanations of these interesting phenomena.

The genus *Pyronema* includes several species, which, as the name implies, commonly inhabit burnt places. The occurrence of the plants of this genus on burnt ground is sufficiently common

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to have suggested the name of the genus, but no one has apparently considered the matter of sufficient importance to warrant investigation.\* Since the most common species of the genus, *Pyronema omphalodes* (Bull.) Fuckel, is one of the few discomycetous fungi in which sexual reproduction has been demonstrated, numerous papers have been written on this phase of the subject, but in each case the matter of the occurrence of the species is dismissed with a simple statement of the fact. Nor, so far as known, has anyone taken the trouble to cultivate the species under artificial conditions either for the study of reproductive processes or in the attempt to gain information as to the reasons for its common occurrence on burnt ground.

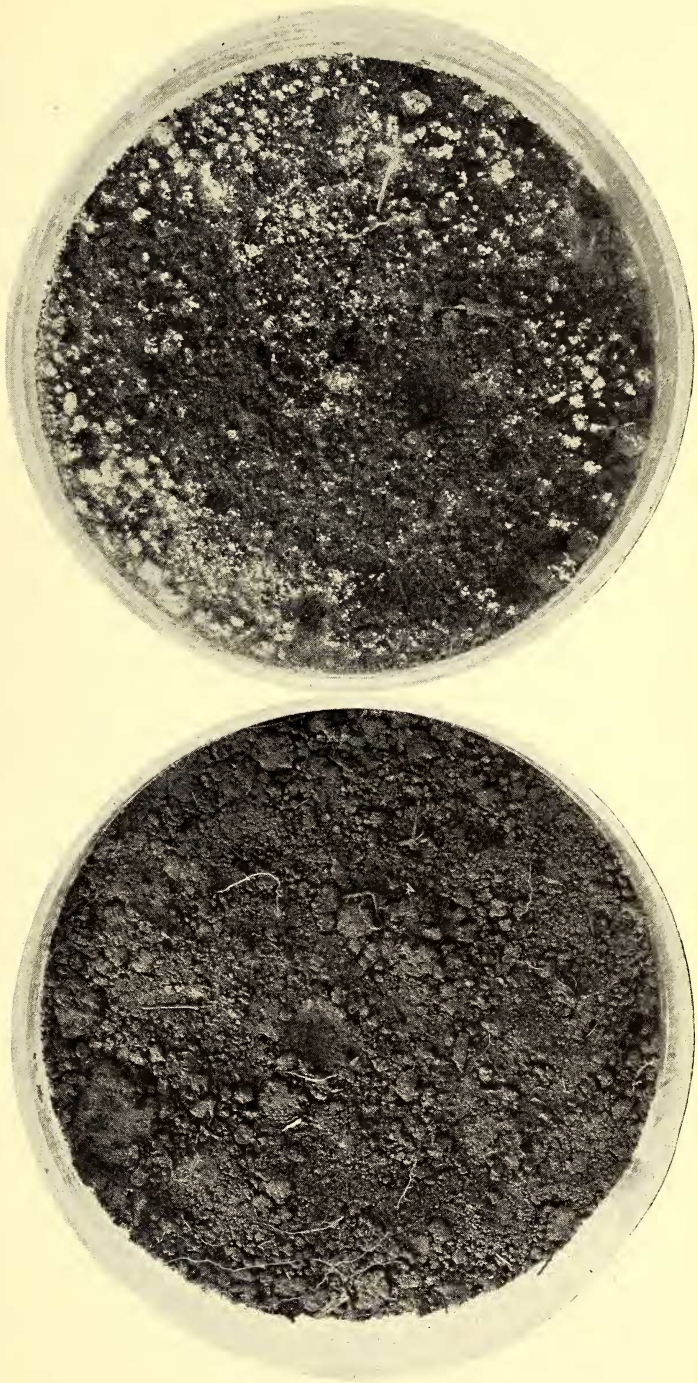
The plants of this genus were first encountered by the writer in 1904, when the above-named species was found to be very common on burnt places near Iowa City, Iowa. Scarcely a burnt place could be visited in and about woods in wet weather on which this species was not found to be present and often in abundance, the plants appearing on charcoal and ashes and the burnt-over soil. While the individual plants are small, ranging from one to two millimeters in diameter, they commonly occur in dense confluent masses often covering a space of several inches, and by reason of their bright color they might, in spite of their small size, be counted among the more attractive forms of fungi.

The second occurrence of this species to attract the attention of the writer was during the fall of 1906 in the propagating houses of the New York Botanical Garden, where it was found to appear on soil sterilized with steam under a pressure of ten to fifteen pounds. Here the plants occurred as usual, forming rose-colored or salmon-colored sheets over the surface of the soil, the groups of plants being surrounded by a cobweb of mycelium. Under these conditions the plants seem to thrive for a time, but

\* Since this paper went to press a synopsis of the article below has come to the attention of the writer showing that some of the conclusions drawn in the present paper have been previously arrived at. Although the present work was conducted without knowledge of this previous work and the line of experimentation is different, the conclusions, so far as the work has gone, are almost identical.

Kosaroff, P. Beitrag zur Biologie von *Pyronema confluens* Tul., gleichzeitig ein Beitrag zur Kenntniss der durch Sterilisation herbeigeführten Veränderungen des Bodens. Bot. Zeit. 66: 23. 1908.

PLATE X



MYCOLOGIA

SOIL CULTURES OF PYRONEMA OMPHALODES





finally mature their fruit and disappear. The species was said to occur on soil sterilized in this manner almost without exception and had been noted for several years past by those carrying on experimental work here requiring the sterilization of soils; but as the fungus usually appeared before seeds had germinated and apparently did no harm, it did little more than to arouse a passing interest. The attention of the writer was at length called to this fungus and it was identified as *Pyronema omphalodes* (Bull.) Fuckel. The occurrence of a fungus commonly associated with burnt places on soil sterilized with steam was a fact of unusual interest, since it indicated that charcoal and carbonaceous materials are not necessary to the life of this fungus as was previously supposed.

In trying to explain these facts it at once became apparent that the high temperatures to which the substrata had been subjected had something to do with the appearance of these plants under the above conditions, but whether the high temperatures had some relation to the spores of the fungus itself in stimulating them to germination or to the substrata only in preparing it for the growth of the fungus was at that time a question.

During the summer of 1907 the species was again observed in North Dakota, where it occurred on bare soil by roadsides where there was no trace of charcoal, but in places which it is easy to suspect had been fire-swept or subjected to considerable temperatures by the heat of the sun and natural conditions of sterilization.

The last appearance of these plants and the one which has prompted the study of the problem which has been made the basis of the present paper was in agar which had been inoculated with the spores of other fungi in the laboratories of the New York Botanical Garden. The appearance of this fungus, uninvited, in three different cultures at the same time in a laboratory where to my knowledge none of the plants of the genus had been studied, even from dried material, for more than two years was sufficiently mysterious to arouse interest.

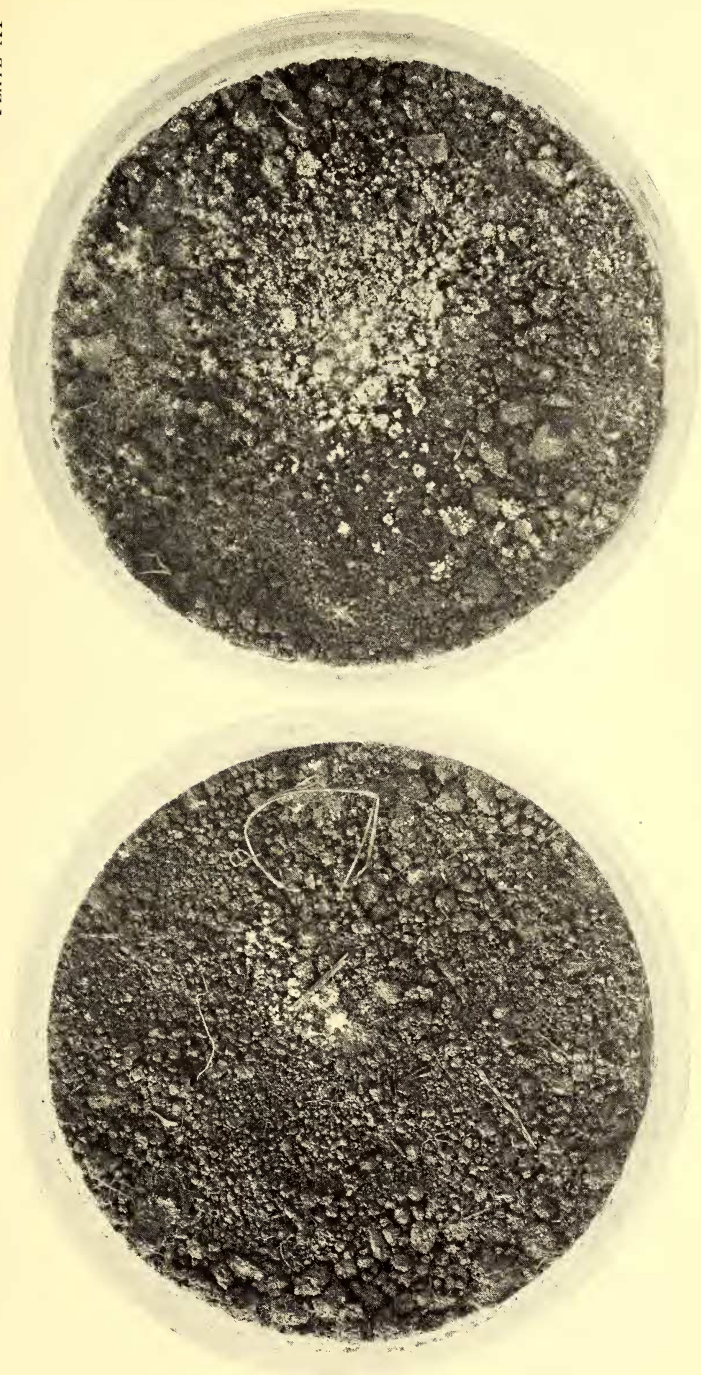
There were two possible explanations of the appearance of this fungus at this time; one that the cultures had become inoculated with the spores from the air and the other that the spores were present in the cultures and had withstood the process of steriliza-

tion. That the spores might not only be able to withstand the process of sterilization, but might even be stimulated to germination by high temperatures was suggested, since it is claimed that the spores of some of the coprophilous fungi must be subjected to the body temperature and other influences of the alimentary canal of animals in order to induce their germination.

In order to test the matter of the effect of heat on the spores of the fungus, mature ascospores were heated to various temperatures and later planted in hanging drop cultures. The heating was accomplished both with dry heat and by heating in a drop of water. In no case could the spores which had been heated to any considerable temperature be made to germinate. On the other hand, mature ascospores which had not been heated germinated readily in drop cultures, proving that if high temperatures have anything to do with the appearance of this fungus the effect is on the substratum only, the spores themselves being as sensitive to heat as are those of other species of fungi.

This species is not sufficiently common to expect that the air of the laboratory is saturated with the spores at all times, but from later experiments it is evident that these cultures were inoculated from the air. The fact that the fungus occurred in cultures in which the agar had been poured over filter paper previously heated to 110° C. for purposes of sterilization again raised the question of the relation existing between this fungus and the heating of the substratum. Cultures of agar were later tried, leaving out the filter paper, and the fungus was found to grow fully as well as in the preceding case. The luxuriant growth of this species on agar is evidence that high temperatures are not necessary in all cases to its growth. Soils sterilized with dry heat require a higher temperature to bring about favorable conditions for the growth of this fungus than are necessary for the sterilization of agar.

From our own observations and experiments there is little doubt that this fungus occurs on burnt places as a result of sterilization of such places by fire. However, it is probable that sterilization means much more than the simple elimination of competition by the destruction of bacteria and other fungi present in the soil. The nature of the changes brought about in soil by heating



SOIL CULTURES OF PYRONEMA OMPHALODES





to high temperatures is a question concerning which little is known and one which is of vital importance to the problem under consideration. While the heating of the soil destroys the fungi already present, there is every reason to believe that it prepares the way for the growth of those species which may be introduced subsequent to sterilization. The experimental work of the present paper has been based mainly on the one species, *Pyronema omphalodes* (Bull.) Fuckel, but the question of the effect of the heating of soils on the production of fungi is doubtless a large one and it is the intention of the writer to extend these investigations to other forms when fresh material can be secured for experimental work.

Some of the observations in support of the above conclusion are: in its occurrence on sterilized soil the fungus usually appears at a very early date and is mature before other forms of vegetation have had time to make any considerable growth; after maturing one crop of ascocarps the fungus gradually disappears, indicating that the most favorable time for its growth is immediately after sterilization; soil sterilized, moistened and allowed to stand for a week appears to be as unfavorable for the growth of *Pyronema* as soils which have never been sterilized, notwithstanding the fact that the soil is entirely free from other forms of vegetation so far as the eye can detect.

In its occurrence in nature on burnt ground, no notes have been made as to the relative time between the burning of the substratum and the appearance of the fungus. This would doubtless depend upon conditions of moisture. A place having been thoroughly sterilized would remain so until the return of moisture, when the *Pyronema* avails itself of the favorable conditions of sterilization and moisture and matures its crop of spores. So far as can be recalled, this species has been found on burnt places only when bare and apparently devoid of other forms of vegetation, indicating that it appears soon after burning or soon after the return of moisture to the burnt places.

In its occurrence on agar, the *Pyronema* grows rapidly, covering the surface of the agar in a three-inch petri dish in about four days. All of the cultures have been slightly contaminated with other fungi in the center of the dish, but fruit has not been